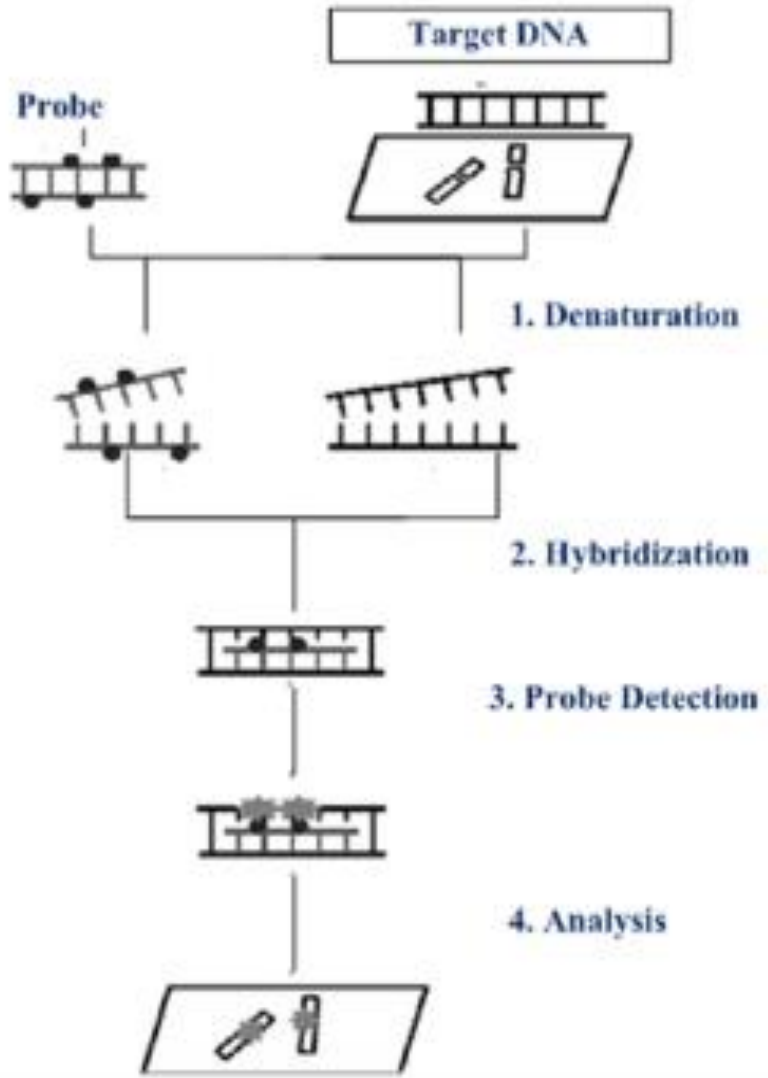
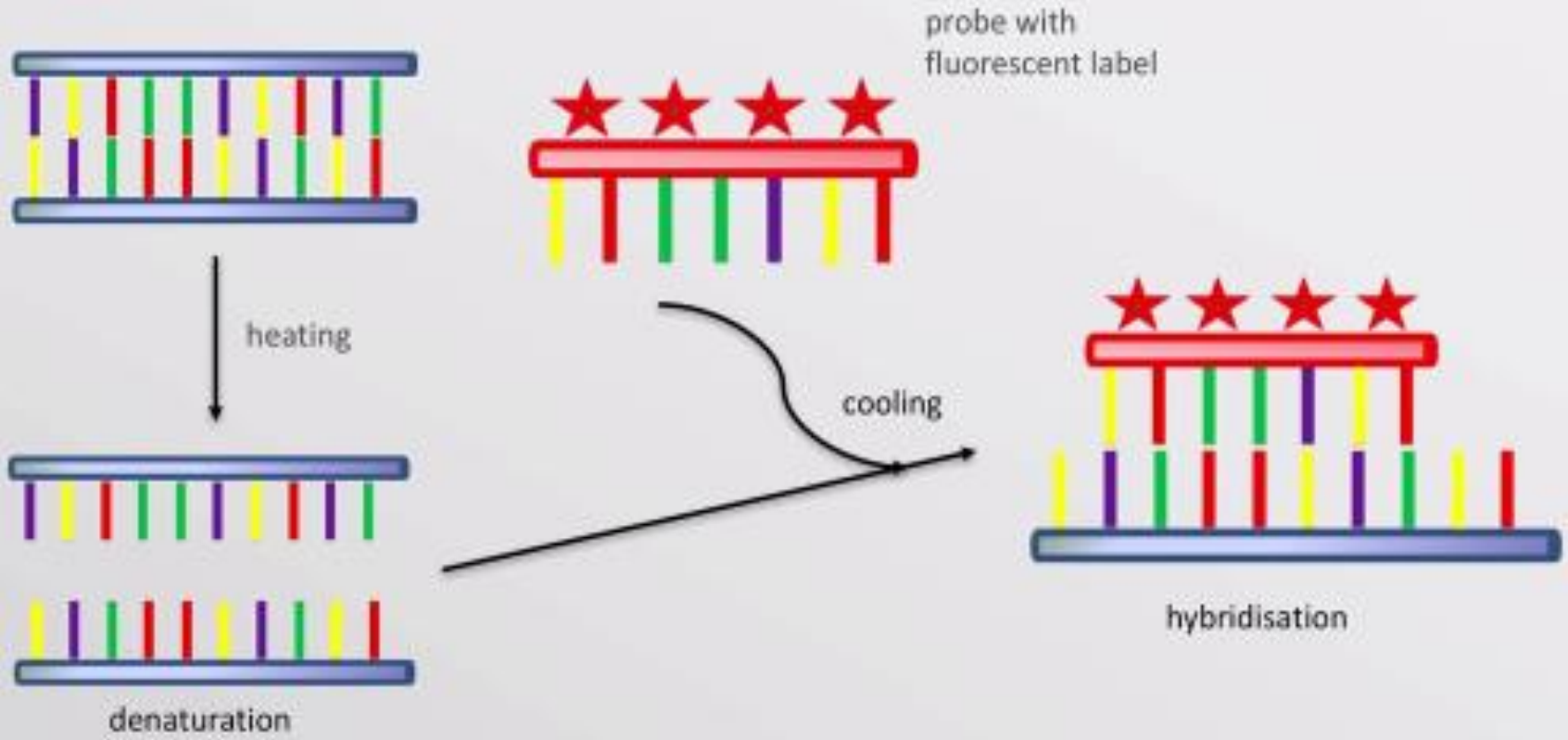
NUCLEIC ACID PROBES

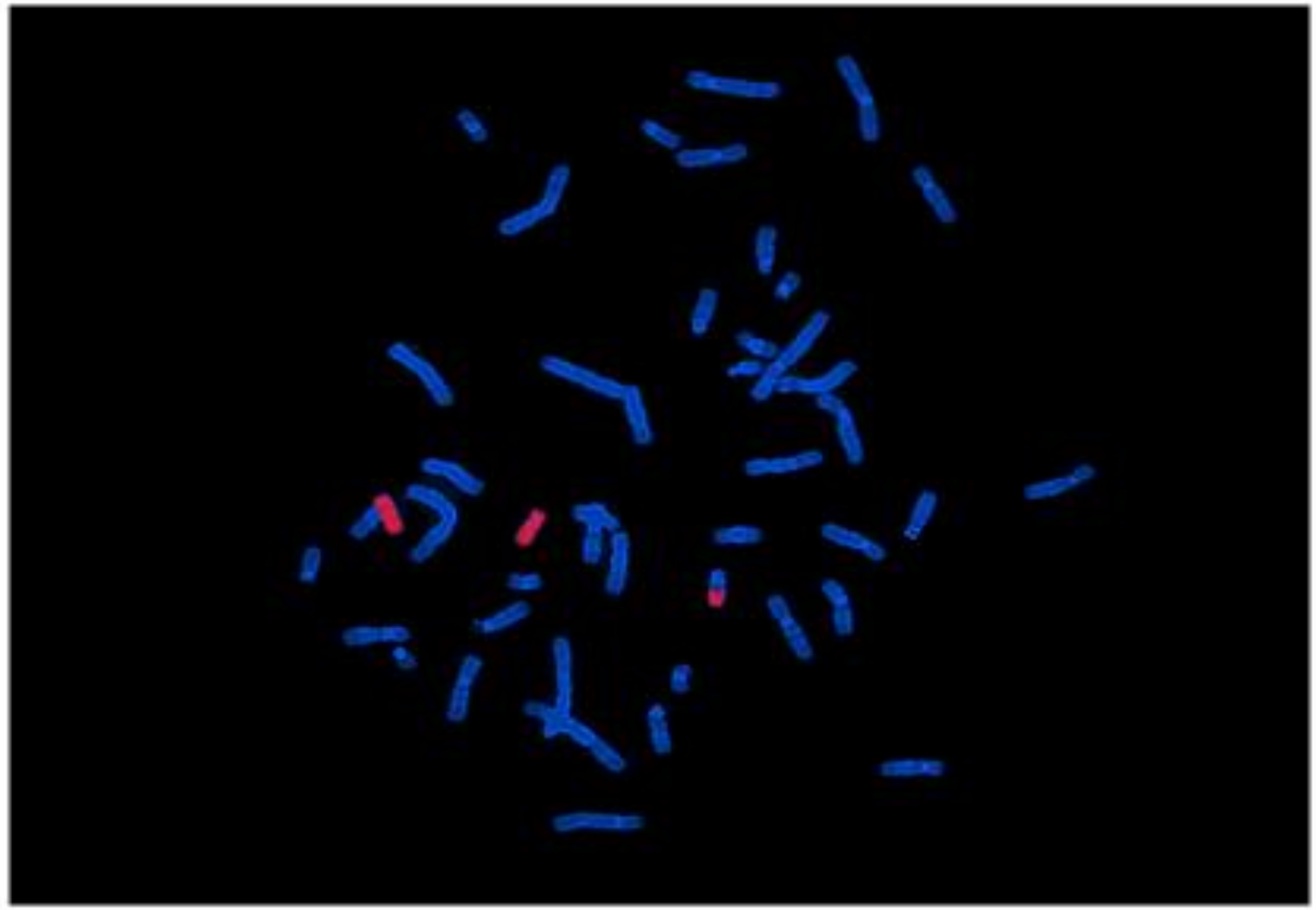


FLUORESCENT IN SITU HYBRIDISATION

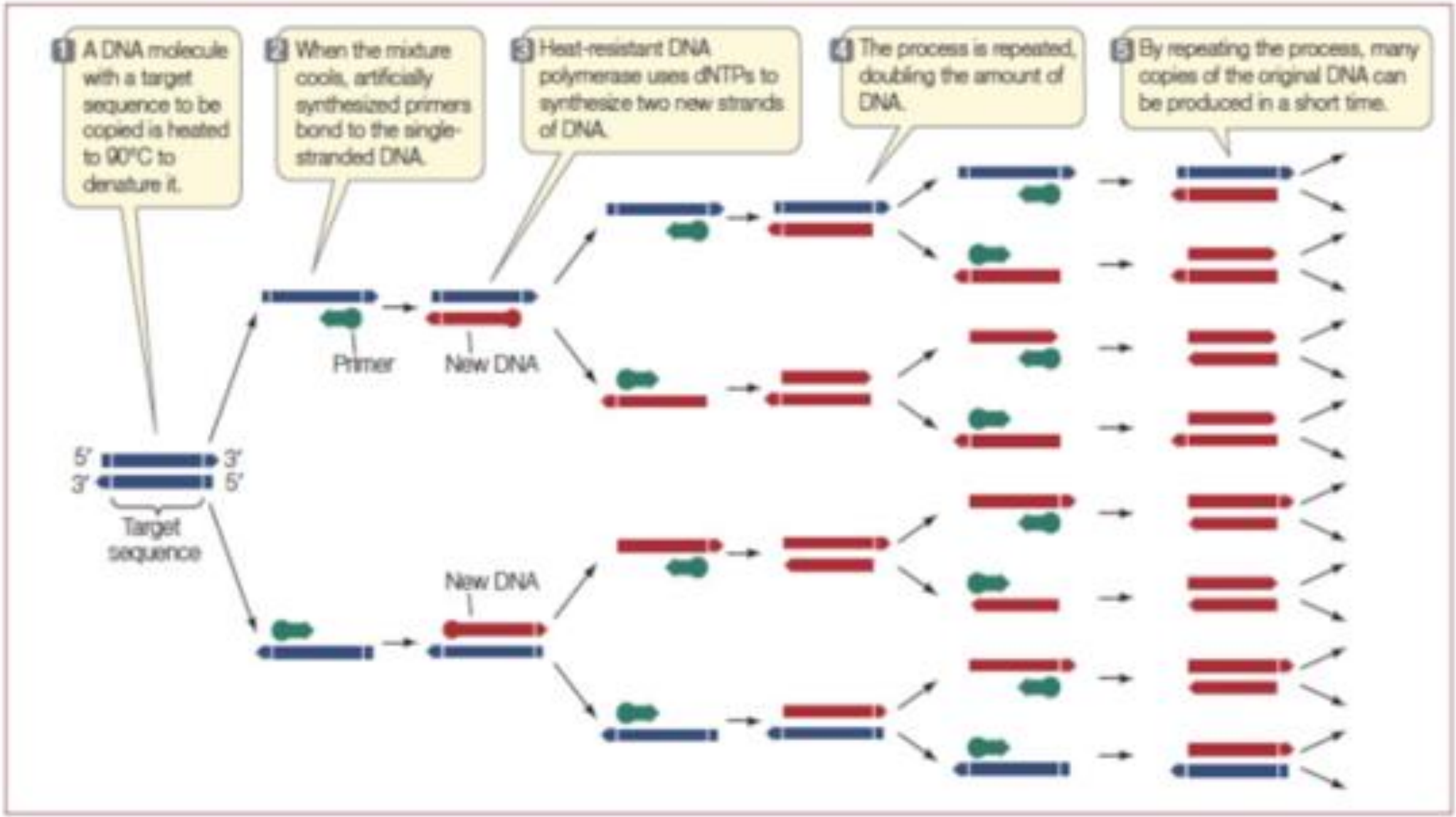
Creative Bioarray

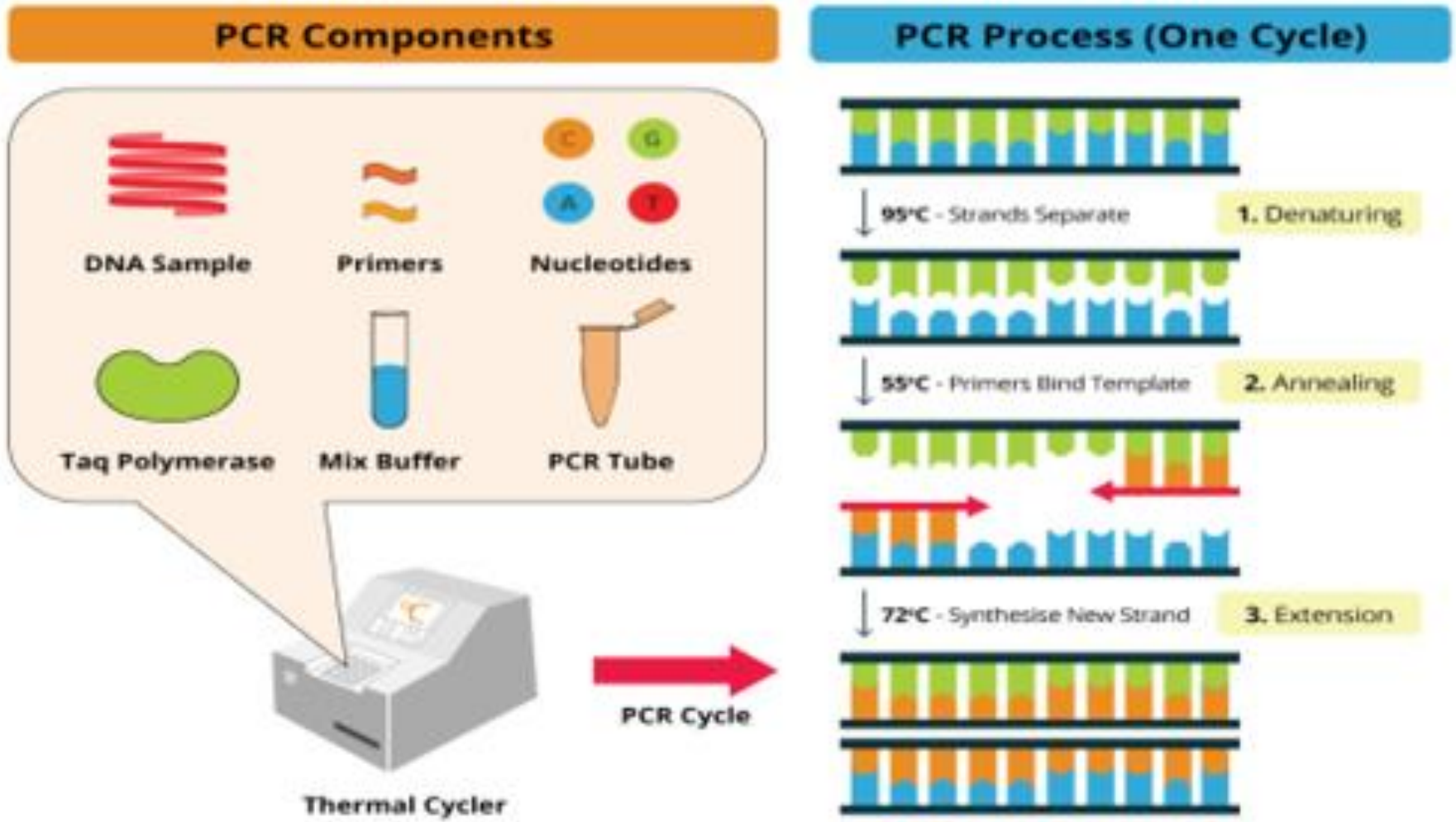
FISH Technology





PCR


1 A DNA molecule with a target sequence to be copied is heated to 90°C to denature it.
2 When the mixture cools, artificially synthesized primers bond to the single-stranded DNA.
3 Heat-resistant DNA polymerase uses dNTPs to synthesize two new strands of DNA.
4 The process is repeated, doubling the amount of DNA.
5 By repeating the process, many copies of the original DNA can be produced in a short time.
5′
3′
3′
5′
Target sequence
Primer
New DNA
New DNA


PCR Components
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq Polymerase
Mix Buffer
PCR Tube
Thermal Cycler
PCR Cycle
PCR Process (One Cycle)
95°C - Strands Separate
1. Denaturing
55°C - Primers Bind Template
2. Annealing
72°C - Synthesise New Strand
3. Extension

What is probe?????

- A probe is normally a short sequence of nucleotide bases that will bind to specific regions of a target sequence of nucleotides.
- The degree of homology between target and probe results in stable hybridization.

??????????????

- "For diagnostic tests, the agent that is used to detect the presence of a molecule in the sample".
- "A DNA sequence that is used to detect the presence of a complementary sequence by hybridization with a nucleic acid sample

probe size

- Probes can range in size from as short as 10 nucleotide bases (molecular weight of 3,300) to as long as 10,000 bases or more (molecular weight of 3,300,000).

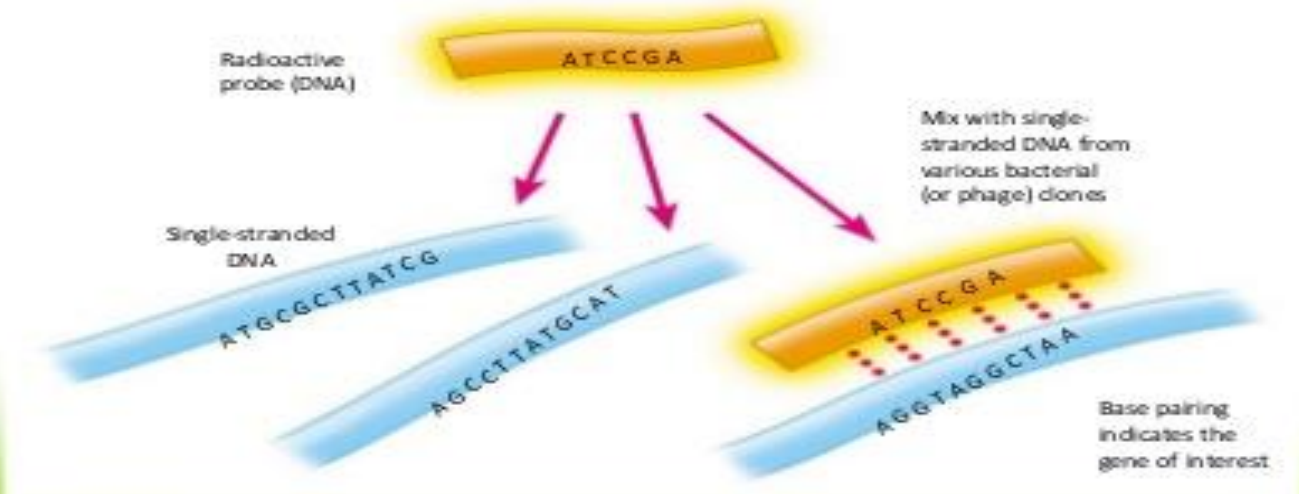
- The most common size range for most probes is between 14 and 40 bases . For statistical uniqueness, a minimum of 20 nucleotide bases are usually needed for a probe.

Which probe is best????
Short or long?????

- Short probes tend to hybridize nucleic acids at very high rates (in minutes).
- whereas longer probes may require reaction times of hours to achieve a stable hybridization.
- short probes do have some disadvantages.
- They are subject to more nonspecific hybridizations, are limited in specificity, and are more difficult to label.
- Long probes hybridize more stably than short probes at high temperatures and low salt concentrations(low stringency).

– A nucleic acid probe

- Is a short, single-stranded molecule of radioactively labeled or fluorescently labeled DNA or RNA



Specificity of the probe

- The base sequence of the probe and the conditions under which the probe is used determine its specificity.

PROBE LABELS

- Radiolabels
- Non-radioactive labels
- Chemiluminescence
- Fluorescence
- Antibodies

Radiolabels

- Probe nucleic acid can be labelled using radioactive isotopes, *e.g.* ^{32}P, ^{35}S, ^{125}I, ^{3}H.
- Detection is by autoradiography or Geiger-Muller counters.
- Radiolabelled probes used to be the most common type but are less popular today because of safety considerations.
- However, radiolabelled probes are the most sensitive, *e.g.* ^{32}P labelled probes can detect single-copy genes in only 0.5 **m**g of DNA.
- High sensitivity means that low concentrations of probe-target hybrid can be detected.

Non-radioactive labels

- These are harmless than radiolabels and do not require dedicated rooms, glassware and equipment or staff monitoring, *etc*. but they are not generally as sensitive.
- Some examples:
- **Biotin** This label can be detected using avidin or streptavidin which have high affinities for biotin.
- **Enzymes** The enzyme is attached to the probe and its presence usually detected by reaction with a substrate that changes colour. Used in this way the enzyme is sometimes referred to as a "reporter group".
- Examples of enzymes used include alkaline phosphatase and horseradish peroxidase.

Chemiluminescence

- In this method chemiluminescent chemicals attached to the probe are detected by their light emission using a luminometer.

- **Fluorescence** Chemicals attached to probe fluoresce under UV light. This type of label is especially useful for the direct examination of microbiological or cytological specimens under the microscope – a technique known as fluorescent *in situ* hybridization (FISH).

Antibodies

- **Antibodies** An antigenic group is coupled to the probe and its presence detected using specific antibodies. Also, monoclonal antibodies have been developed that will recognize DNA-RNA hybrids.

BRINGING PROBE AND TARGET TOGETHER

- Probe and target sequence hybridize with each other but how this is brought about can vary. There are 4 main formats:

1. Solid support

- Target (usually) is bound to a solid support such as a microtitre tray or filter membrane.
- Probe is added in solution and binds to target (if present) on solid support.
- After washing to remove unbound probe, hybridization is detected on the solid support using whatever method is appropriate for the probe label.

2. In solution

- Both the probe and the target are in solution. Because both are free to move, the chances of reaction are maximized and, therefore, this format is generally faster than others.

3. In situ

- In this format probe solution is added to fixed tissues, sections or smears which are then usually examined under the microscope.
- The probe label, *e.g.* a fluorescent marker, produces a visible change in the specimen if the target sequence is present and hybridization has occurred.
- However, the sensitivity may be low if the amount of target nucleic acid present in the specimen is low. This can be used for the gene mapping of chromosomes, and for the detection of microorganisms in specimens.

probes have 3 basic applications in medicine:

1. **Detection of specific nucleic acid sequences**

Probes can be especially useful for detecting microorganisms that grow slowly (*e.g. Mycobacterium tuberculosis*) or which cannot be cultured on artificial growth media (*e.g.* all viruses).

- **Examples of the Applications of Nucleic Acid Probes in Medical Research**
- Detection of tumor suppressor genes in human bladder tumors
- Identification of *Leishmania* parasites
- Detection of malignant plasma cells of patients with multiple myeloma
- Diagnosis of human papillomavirus
- Visual gene diagnosis of HBV and HCV
- Detection and identification of pathogenic *Vibrio parahaemolyticus*
- Detection of *Vibrio cholerae*
- Molecular analysis of tetracycline resistance in *Salmonella enterica*
- Identification of fimbrial adhesins in necrotoxigenic *E. coli*
- Epidemiological analysis of *Campylobacter jejuni* infections
- Molecular analysis of NSP4 gene from human rotavirus strains
- Physical mapping of human parasite *Trypanosoma cruzi*
- Detection and identification of African trypanosomes
- Detection and identification of pathogenic *Candida* spp.
- Identification of *Mycobacterium* spp.